Anti-Gliadin ELISA Kit

Cat. No.:DEIA1971
Pkg.Size:96T

Intended use

Anti-Gliadin is an indirect solid phase enzyme immunoassay (ELISA) for measurement of IgG and IgA class autoantibodies against Gliadin in human serum or plasma.

General Description

Gliadin is the main component of gluten, which occurs in wheat and other domestic grain types like rye, barley and oats, and may lead to severe diseases of the intestinal mucosa in sensitive children and adults. Celiac disease, a gluten-induced enteropathy, appears rather frequently (1 case on 300 births) and is a typical example of a non-IgE mediated food allergy. Genetically, histocompatibility antigens on the chromosome 6 are responsible for the disease. Celiac disease manifests itself practically as a constant reaction against gliadin. By the toxic effect of gluten in the intestinal tract, antibodies, cytokines and lymphocytes are released, which lead to internal lesions and inflammations. Further, the microvilli of the intestine are almost completely reduced, so that the inner intestinal surface becomes flat. The resulting malabsorption leads to a deficit of above all trace elements and vitamins. Loss of weight, diarrhea, flatulence and abdominal pain are observed as symptoms. An invasive diagnostic possibility represents the biopsy of the intestinal mucosa. In addition serological methods for the determination of IgG and IgA antibodies against gliadin, reticulin and endomysium in the patient serum are increasingly used as a screening method. For children with a gluten-sensitive enteropathy, the incidence was calculated to 90-100%, for adults with celiac disease 75-90% and for dermatitis herpetiformis 40-50%. Elevated levels of IgA anti-gliadin demonstrate an active process and are in close correlation with a villous atrophy in children.

Principle Of The Test

Purified gliadin from wheat is bound to microwells. Antibodies to this antigen, if present in diluted serum, bind in the microwells. Washing of the microwells removes unbound antibodies. Horseradish peroxidase (HRP) conjugated anti-human IgG and anti-human IgA immunologically bind to the specimen sample antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG and IgA antibodies present in the original sample.

Reagents And Materials Provided

1. Divisible microplateconsisting of 12 modules of 8 wells each, coated with Gliadin. Ready to use.
2. combined Calibratorswith IgG and IgA class Anti-Gliadin antibodies (A-F) in a serum/buffer matrix (PBS, BSA, NaN3<0,1% (w/w)) containing: 0; 6.3; 12.5; 25; 50; and 100 U/ml. Ready to use; 6 vials, 1.5 ml each
3. Anti- Gliadin Controlsin a serum/buffer matrix (PBS, BSA, NaN3<0.1% (w/w)) positive and negative , for the respective concentrations see the enclosed QC insert. Ready to use; 2 vials, 1.5 ml each
4. Sample buffer(Tris, NaN3<0.1% (w/w)), yellow, concentrate (5x); 1 vial, 20 ml
5. Enzyme conjugatesolution (PBS, Proclin 300 <0.5% (v/v)), (light red) containing polyclonal rabbit anti-human IgG and anti-human IgA; labelled with horseradish peroxidase. Ready to use; 1 vial, 15 ml
6. TMB substratesolution. Ready to use; 1 vial, 15 ml
7. Stop solution(contains acid). Ready to use; 1 vial, 15 ml

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8. Wash solution(PBS, NaN3<0.1% (w/w)), concentrate (50x); 1 vial, 20 ml

Materials Required But Not Supplied

1. Microplate reader capable of endpoint measurements at 450 nm
2. Multi-Channel Dispenser or repeatable pipet for 100 µl
3. Vortex mixer
4. Pipets for 10 µl, 100 µl and 1000 µl
5. Laboratory timing device
6. data reduction software
7. distilled or deionized water
8. graduated cylinder for 100 and 1000 ml
9. plastic container for storage of the wash solution

Storage

1. Store the kit at 2-8 °C.
2. Keep microplate wells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage and usage.
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2-8 °C.

Specimen Collection And Handling

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8 °C for up to five days or stored at -20 °C up to six months.
5. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
6. Testing of heat-inactivated sera is not recommended.

Reagent Preparation

Preparation of sample buffer

Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 ml prior to use.

Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use.

Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Sample preparation

Dilute all specimen samples 1:100with sample buffer before assay.

Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well.

Controls are ready to use and need not be diluted.

Assay Steps

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted specimen samples.

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2. Pipet 100 µlof calibrators, controls and prediluted specimen samples in duplicate into the wells.
3. Incubate for 30 minutes at room temperature (20-28 °C).
4. Discard the contents of the microwells and wash 3 times with 300 µlof wash solution.
5. Dispense 100 µlof enzyme conjugate into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with 300 µlof wash solution.
8. Dispense 100 µlof TMB substrate solution into each well.
9. Incubate for 15 minutes at room temperature.
10. Add 100 µlof stop solution to each well of the modules and incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.

The developed colour is stable for at least 30 minutes. Read optical densities during this time.

Automation

The Anti-Gliadin ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

Precautions

1. Do not interchange kit components from different lots.
2. Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
3. Avoid contact with the TMB (3,3´,5,5´-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
4. Avoid contact with the Stop Solution which is acid. If it comes into contact with skin, wash thoroughly with water and seek medical attention.
5. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide (NaN3) is highly toxic and reactive in pure form. At the product concentrations, though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 8., 9., 10.).
6. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
7. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
8. Do not pipette by mouth.
9. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
10. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

Analyte Gene Information

Gene Name	LOC543191 alpha-type gliadin [Triticum aestivum]
Official Symbol	LOC543191
GeneID	543191

REFERENCES

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